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BIOASSAY AND CHEMICAL ANALYSES OF THE EMISSIONS FROM RAPESEED ETHYL AND METHYL ESTER BIODIESEL FUEL

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BIOASSAY AND CHEMICAL ANALYSES OF THE EMISSIONS FROM RAPESEED ETHYL AND METHYL ESTER BIODIESEL FUELS

FINAL REPORT

BY

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I. SUMMARY

Diesel-powered vehicle emissions are a major source of particulate matter from motor vehicles. Particles smaller than 10 μm (PM10) aerodynamic diameter and typically submicron in size can penetrate the deepest portions of the human lung and can lead to adverse health effects such as respiratory problems and death. Particulate matter in diesel exhaust is considered a probable human carcinogen and contains a complex mixture of toxic compounds.

The emissions from heavy-duty diesel engines contain both particulate and vapor-phase associated compounds. The vapor-phase consists of volatile and semi-volatile compounds, including aldehydes and polycyclic aromatic hydrocarbons (PAH), many of which are carcinogenic to animals or suspected to be carcinogenic to humans.

The volatile compounds exert a relatively high vapor pressure at room temperature (for example, dichloromethane = 440 torr at 25°C) and non-volatile compounds exert low vapor pressures (for example, pyrene = 6.85×10^{-7} torr at 20°C). As the boiling point of a compound decreases, its vapor pressure increases. As the vapor pressure increases, there is a greater tendency for a compound to evaporate, or volatilize. The semi-volatile compounds have vapor pressures and boiling points between those of volatile and non-volatile compounds. Due to their volatility, vapor-phase compounds require special experimental approaches for sampling, extracting and mutagenicity testing in order to minimize sample loss. Although many toxicological studies have focused on diesel particulates, few have focused on those toxic compounds emitted from the combustion of biodiesel fuel.

We investigated the particulate- and vapor-phase emissions from a medium-duty diesel engine using rapeseed ethyl ester (REE) and methyl ester (RME) biodiesel fuels and compared these emissions to those obtained using 100% diesel (2-D) fuel. Emission samples were collected at Southwest Research Institute (SwRI) under conditions controlled with an engine dynamometer and dilution tunnel (a facility where engine exhaust is diluted, collected and measured). The EPA Heavy-Duty Diesel Engine Federal Test Protocol was used. Particulate- and vapor-phase emission samples were collected for chemical and bioassay analyses using a Teflon filter and polyurethane foam (PUF) adsorbent in series. Solvent extracts from the particluate- and vapor-phase emission samples were chemically analyzed for polycyclic aromatic hydrocarbons (PAHs) using gas chromatography/mass spectrometry (GC/MS) analyses. For the particulate-phase, PAH emission rates (µg PAH/brhp-hr) were determined. The vapor-phase emissions that were trapped on the PUF adsorbents were extracted using a supercritical fluid extraction (SFE) procedure and the extracts were analyzed by GC/MS. Bioassay analyses were conducted using the *Salmonella* microsuspension assay for both the particulate- and vapor-phases and mutagen emission rates (revertants/brhp-hr) were determined.

For the PM samples, emission rates for benz(a)anthracene and chrysene were lower for the 100% REE fuel than for the 100% 2-D fuel, with or without the catalyst. Benzo(b)fluoranthene and benzo(k)-fluoranthene had similar emission rates for the 100% REE, 50% REE, and 100% 2-D fuels. There were higher emissions of benzo(e)pyrene, benzo(a)pyrene, and benzo(g,h,i)perylene for the 100% REE than for the 100% 2-D fuel. The emission rate for benzo(e)pyrene, benzo(a)pyrene, and benzo(g,h,i)perylene were typically in the sub-microgram per brhp-

hr, and the results are similar to those reported by SwRI. For the vapor-phase PAHs collected on polyurethane foam (PUF), there was in general, an increasing gradient of PAH emissions (naphthalene through phenanthrene) from 100% REE to 50% REE to the 100% 2-D fuel. There were also high levels of selected high molecular weight, or "heavy" PAHs present in the PUF samples that are typically found only in the particulate-phase. The source of these heavy PAH compounds present in the PUF samples was uncertain, but could be due to PAH standards added before sampling or chance contamination.

The particulate extracts were analyzed with a modified Salmonella mutagenicity assay (microsuspension assay). The 100% REE and 100% RME had mutagen emission rates that were approximately three times less than those for the 100% 2-D fuel. The mutagen emission rates for the blends of biodiesel with diesel were between the rates for the 100% biodiesel and 100% 2-D fuels. In general, the biodiesel fuels had lower emissions of mutagenic compounds than the conventional diesel fuel.

II. INTRODUCTION

A. Statement of Problem

The measurement of human exposure to airborne particulates is important because particles that are 10 μm or less (PM10) can enter and deposit on the trachea, bronchi and alveoli (Schlesinger, 1985). These particles have been associated with increased respiratory infections, decreased lung function and morbidity (Pope *et al.*, 1992; Dockery *et al.*, 1992). These particles are also known to contain numerous carcinogenic and mutagenic compounds (Daisey, 1987; Sheldon *et al.*, 1992; Atkinson et. al., 1988). Although there are numerous human-caused outdoor sources of PM10 in the environment, one major source is from the operation of heavy-duty diesel vehicles.

The toxic compounds associated with PM10 from diesel emissions are difficult to routinely characterize chemically due to the complex mixture of compounds. Further, additives or changes in fuel composition can alter the toxic emissions from these sources. Biodiesel fuels are produced from vegetable oils, such as rapeseed and soybean, and are currently being evaluated as alternatives to conventional diesel fuel. Use of a biodiesel fuel is believed to reduce the emission of toxic compounds, but studies are needed to test this hypothesis.

In the present study, particulate- and vapor-phase samples were collected from biodiesel and diesel emissions produced by a medium-duty diesel engine at Southwest Research Institute (SwRI). The particulate- and vapor-phase samples were extracted and analyzed with integrated bioassay and chemical techniques at UC Davis (UCD).

B. Goals and Objectives

The overall objective of this project was to use integrated methodology to chemically characterize the particulate- and vapor-phase emissions from a medium-duty diesel engine exhaust using the following specific fuels: 1) 100% rapeseed ethyl ester (REE), 2) 100% rapeseed methyl ester (RME), 3) a 50% blend of REE with diesel fuel, 4) a 50% blend of RME with diesel fuel, 5) a 20% blend of REE with diesel fuel , 6) a 20% blend of RME with diesel fuel, and 7) 100% diesel fuel (2-D). The diesel fuel used in these tests and blends was certification-grade Phillips low-sulfur diesel fuel. The current work reported herein had the following specific goals:

1. Obtain particulate- and vapor-phase emission samples from a medium-duty diesel engine using biodiesel fuels, biodiesel fuels blended with diesel fuel, and diesel fuel.

2. Prepare particulate- and vapor-phase samples for biological and chemical analyses using both SFE and conventional solvent extraction techniques.

3. Test all sample extracts with a bioassay. Determine specific mutagenic activity of particulate- and vapor-phase emission sample extracts using bioassay.

4. Develop bioassay-derived emission rates for each fuel type.

5. Determine PAH emission rates for each fuel type.

6. Submit draft final and final reports.

C. Background

The exhaust from diesel engines has been determined by the International Agency for Research on Cancer to be a probable human carcinogen (IARC, 1989). In a number of animal studies, whole diesel exhaust was carcinogenic (IARC, 1989). Bagley *et al.* (1987) investigated the effect of ceramic particle traps on the chemical mutagens present in diesel exhaust. In their study, the mutagenic activity in both the soluble organic fraction of PM and volatile organic compounds of diesel exhaust was measured. The samples were collected in a dilution tunnel from an 8-cylinder medium-duty diesel engine. A number of compounds were tentatively identified in both phases which included PAHs such as fluorenone, anthracene/ phenanthrene, ketones, and methyl anthracene/phenanthrene.

Much of the experimental work regarding diesel exhaust has been on PM. A number of potent mutagens have been isolated and identified using the microsuspension assay in conjunction with chemical analysis (Scheutzle *et al.*, 1982; Lewtas, 1988). Many of these compounds are suspected human carcinogens.

A few investigators have reported the potential carcinogenicity of the vapor-phase. For example, Iwai *et al.* (1986) conducted chronic inhalation studies in rats that were exposed to unfiltered and filtered diesel exhaust. The primary cause of death among rats exposed only to the vapor-phase compounds present in diesel exhaust was malignant lymphoma, and the incidence was statistically significant compared to controls (Iwai, *et al.*, 1986).

In a major animal exposure study, Mauderly *et al.* (1994) exposed rats to emissions from a light-duty diesel engine or carbon black. The carbon black particles were similar to the soot particles in the diesel engine exhaust, but they contained markedly lower amounts of adsorbed organic compounds. The investigators found that both the carcinogenic and noncarcinogenic effects produced by prolonged exposure to diesel engine exhaust were nearly identical to the effects produced by lamp black in the strain of rats used. The investigators concluded that, in this type of dosing regimen, the organic fraction may not be the cause of the observed effects and that mechanisms by which inhaled diesel soot and carbon black cause lung neoplasms in rats remain undefined.

Short-term tests for mutagenicity can be used to screen for the mutagenic components of complex environmental mixtures such as diesel exhaust. Mutagenic activity, as determined by short-term bioassays in cells in culture or in whole animal studies, indicates that there has been damage to DNA, the principal genetic material in all living organisms. Mutagenic activity, as determined in *Salmonella typhimurium* as the indicator organism, indicates that DNA damage has occurred in these cells. The mechanism that leads to cancer in humans and animals is thought to be the result of a complex and multi-staged process that involves mutation of DNA in specific genes (Sugimura *et al.*, 1992). Most of the chemicals that are known to be carcinogenic to humans also damage DNA.

The *Salmonella*/microsome mutagenicity assay, commonly known as the Ames test (Ames *et al.*, 1975), is the most widely used and validated of all assays for mutagenicity. The test has been very

useful in directing chemical identification of mutagenic compounds present in environmental mixtures (Lewtas, 1988). Previously, Kado *et al.* (1983, 1986) developed a simple modification of the Ames test, known as the microsuspension assay. This assay is 10 to 20 times more sensitive than the standard Ames test for a given amount of mutagen tested.

Westerholm *et al.* (1991) studied the chemical composition and mutagenicity of exhaust from a heavy-duty diesel vehicle (14.2 L engine) tested during transient driving conditions. The authors used a cryo-trap, XAD-2, and polyurethane foam (PUF) to collect the diluted volatile organic compounds from the exhaust. Polyurethane foam (white polyether and charcoal gray polyester forms), or PUF, has been extensively used in air sampling. Because of its low resistance to air flow, PUF is useful for sampling at high flow rates. PUF has a large sorption surface, is easy to handle and store, and is relatively inexpensive. However, PUF requires thorough pre-cleaning with organic solvents before use. Mutagenic activity was detected on the XAD-2 and PUF samples. The contribution of mutagenic compounds in the semi-volatile phase was 20% in tester strain TA100 (±S9), 10% in TA98 (-S9), and 37% in tester strain TA98 (+S9). A number of 3-ring PAH (substituted and unsubstituted) were tentatively identified in these samples. Phenanthrene and methyl phenanthrene, for example, were the highest emitted PAHs in the particulate phase. The amounts of PAH emitted in the semi-volatile phase was approximately three times higher than that emitted in the particulate phase.

In previous work at UC Davis, we used integrated SFE, mutagenicity testing, and GC/MS analysis to identify vapor-phase

compounds present in diesel exhaust (Kado *et al.,* 1996). SFE is a reliable and efficient technique that can expedite the separation and analysis of complex chemical mixtures (Taylor, 1992; Hawthorne *et al.,* 1988). Supercritical fluids are gases that have many characteristics of a liquid, including the ability to extract compounds from various collection media. Supercritical fluids are used at specific temperatures and pressures and behave as liquids, but they do not generate liquid solvent waste.

Biodiesel fuels are produced from vegetable oils like rapeseed, soybean, sunflower, palm, coconut, and used oils, and are currently being evaluated as alternatives to diesel fuel. The emissions of toxic compounds are thought to decrease with the use of biodiesel fuel, especially those compounds associated with PM.

Few studies have been reported that compare differences in toxic compound emissions associated with PM from diesel and biodiesel fuels. In recent work, Bagley *et al.* (1998) compared emissions from a diesel engine using 100% soy methyl ester biodiesel and diesel fuels, with and without the use of an oxidation catalytic converter (OCC). The authors reported 50 to 80% reductions in total PM for the use of diesel and biodiesel fuels with the OCC, as compared to emissions from the use of diesel fuel without the OCC. Using biodiesel fuel, particulate-associated PAH emissions were reported to be lower, with or without the OCC. Substantial reductions in vapor-phase PAH emissions were also reported when either fuel was used with the OCC. In addition, both particulate- and vapor-phase-associated mutagenicity were reduced by 50%, for both fuels with the OCC.

Sharp *et al.*,1996 summarized SwRI's measurements of regulated pollutants taken during transient cycle testing of biodiesel emissions. The 100% REE and RME biodiesel fuel emissions had less particulate matter, hydrocarbon, and carbon monoxide emissions than the 100% diesel fuel. There were no significant differences in the NOx emissions, except for 100% RME which had a slight increase in NOx over the 100% diesel fuel.

In the present study, we investigated the concentration of PAHs in the particulate- and vapor-phase samples. Emission rates were calculated from these concentrations. We also used bioassay to evaluate the complex mixtures of compounds present in the particulate-phase.

III. Materials and Methods

A. Test Engine and Fuels

1. Test Engine / Facility

The test engine was a 1995 Cummins turbocharged B5.9 liter diesel engine, equipped with an in-line mechanically governed fuel injection system. The engine came supplied with a catalytic converter. The emissions testing was done with and without the catalytic converter attached to the exhaust pipe.

The test engine was located at the Southwest Research Institute (SwRI) emissions testing facility in San Antonio, Texas. The collection of samples was done in conjunction with a test of regulated emissions. (Sharp *et al.*, 1996). The test plan was designed to include three consecutive hot start cycle emission samples collected individually for each test fuel, with and without the catalytic converter attached to the exhaust system.

2. Test Fuels

The biodiesel fuels tested were rapeseed ethyl ester (REE), rapeseed methyl ester (RME), and blends of REE or RME with diesel fuel. The biodiesel fuels were produced and supplied by Dr. Charles Peterson at the University of Idaho. The specific fuels tested were: 1) 100% REE, 2) 100% RME, 3) a 50% REE blend with diesel fuel, 4) a 50% RME blend with diesel fuel, 5) a 20% REE blend with diesel fuel, 6) a 20% RME blend with diesel fuel, and 7) 100% emissions-grade diesel fuel (2-D). The properties of these fuels are presented in a report on regulated and unregulated emissions (Sharp *et al.*, 1996).

The reference diesel fuel was a 1994 emissions-grade 2-D diesel fuel produced by Phillips Petroleum (Lot No. S-946X). The biodiesel fuels were blended with this reference diesel fuel by SwRI as detailed in the final report of Sharp *et al.*, (1996).

B. Test Cycle

The test cycle used throughout was the EPA Heavy-Duty Diesel Engine Transient Test Cycle (CFR40, Pt. 86, Subpt. N) which incorporates both city and highway driving components as illustrated in **Figure 1**.

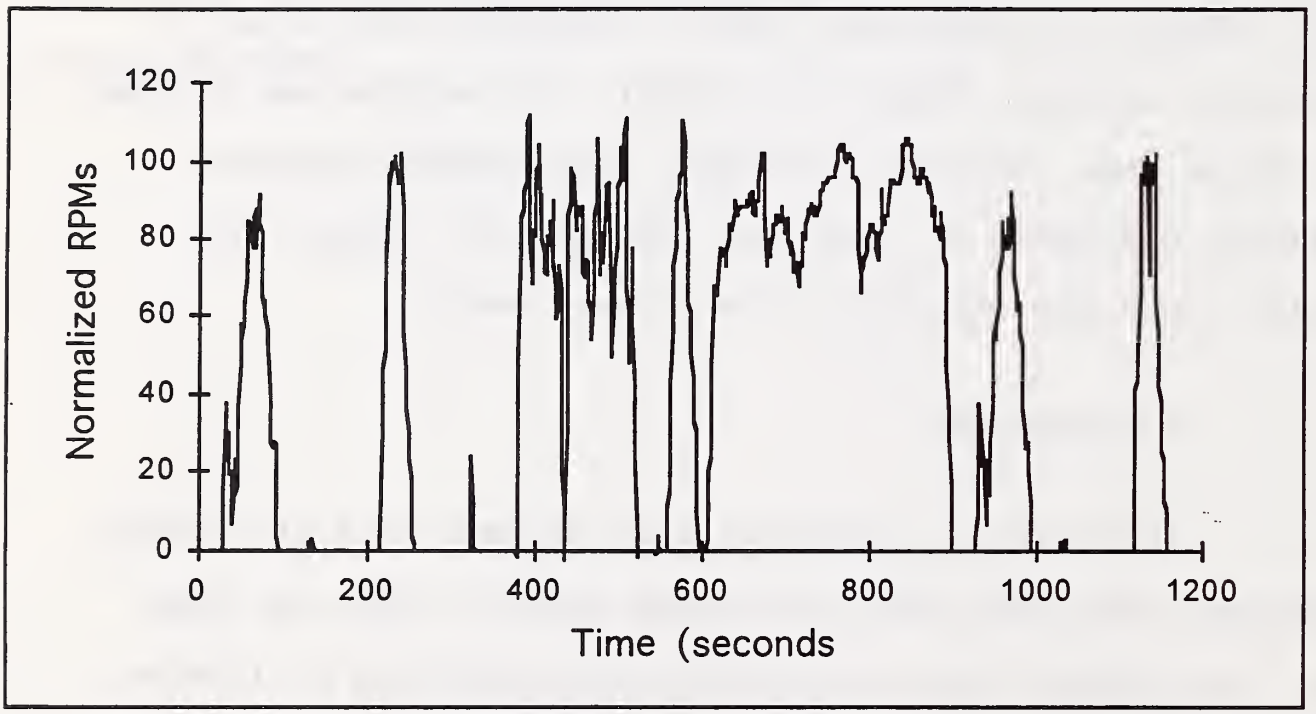

Figure 1. EPA Heavy-Duty Diesel Engine Transient Test Cycle.

C. Sampling

Sampling was conducted by SwRI as part of a study to measure both regulated and unregulated emissions. A portion of their samples (representing complete cycles) were sent to UC Davis for chemical and bioassay analyses. The particulate matter was collected on 20 inch x 20 inch T60A20 fiberfilm filters (Pallflex Corp., Putnam, Conn.). The filters were used without further treatment before sampling. A single filter was placed in the hi-volume sampling unit and the flow rate ranged from 52 to 55 standard cubic feet per minute (scfm). The vapor-phase samples were collected downstream of the filter sample using a pre-cleaned polyurethane foam (PUF) adsorbent placed within a cartridge that was connected to a separate sampling pump (Sharp *et al.,* 1996). Sampling flow rates for the PUF samples were nominally 1.8 scfm.

As previously mentioned, there were 7 fuels tested and the emission samples were collected with and without the catalytic converter attached. This resulted in a combination of 14 sample types that were collected, extracted and analyzed.

D. Chemicals

HPLC grade methanol, acetone, hexane, and water were obtained from Fisher Scientific (Houston, TX). For mutagenicity experiments, benzo(a)pyrene and dimethylsulfoxide (DMSO, spectrophotometric grade) were from Aldrich Chemical Co. (Milwaukee, WI) and were used without further purification. Dichloromethane (DCM, OmniSolve) was from EM Science (Gibbstown, NJ). For solid phase extractions (SPE), acetonitrile (ChromPure) and hexane (UV, High Purity) were from Burdick and Jackson (Muskegon, MI).

Deuterated standards were obtained from Cambridge Isotopes (Andover, MA) and included methyl naphthalene-d_{10}, fluorene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, pyrene-d_{10}, benzo(b)fluoranthene-d_{12}, benzo(k)-fluoranthene-d_{12}, benzo(e)pyrene-d_{12}, dibenz(a,h)-anthracene-d_{14}, and benzo(g,h,i)perylene-d_{12}. Naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12} were from AccuStandard (New Haven, CT).

All other PAH standards except for benzo(e)pyrene and perylene were obtained from Restek (Bellefonte, PA). Benzo(e)pyrene, perylene and the substituted PAHs were obtained from Chem Service (West Chester, PA). PAH standards for gas chromatography/mass spectrometry (GC/MS) calibrations were from the National Institute of Standards and Technology (NIST, Gaithersburg, MD).

E. Bioassay Analyses

Bioassay experiments were conducted to determine the specific mutagenic activity of the particle and vapor-phase extracts. Filter samples were extracted with DCM and sonication. The extracts were filtered through a pre-cleaned Teflon filter (Gelman, CR PTFE, 0.45 μm.) PUF samples were extracted using supercritical carbon dioxide (Kado, *et al.*, 1997). For all samples, the specific mutagenic activity is reported as the number of revertants per mg of particulate matter. The specific mutagenic activity is determined from the slope obtained from the linear portion of the dose-response curve and are used to determine the emission rate of mutagenic compounds present in the exhaust. The bioassay used throughout was the micro-suspension assay previously reported by Kado *et al.*, (1983, 1986). All sample extracts were resuspended in DMSO prior to testing in the bioassay.

F. Chemical Analyses

1. Instrumental conditions and column selection

The filter and PUF extracts for the PAHs were analyzed using a Hewlett-Packard Model 5890 Series II Gas Chromatograph (GC) equipped with a Model 8290 autosampler interfaced to a Hewlett-Packard Model 5970A quadrupole mass selective detector (MSD). The GC was equipped with a split/splitless injector and an electronic pressure controller. The injector was run in the splitless mode and the electronic pressure controller was programmed for vacuum compensation and constant flow mode. The GC was equipped with a 2 m x 0.25 mm id deactivated silica pre-column connected to a

30 m x 0.25 mm i.d. DB-5 fused silica capillary column (0.25 μm film thickness; J&W Scientific). The MSD was run using both the selective ion monitoring (SIM) and electron impact (EI) modes.

The target PAH analytes and the corresponding deuterated internal standards are listed in **Table 1** along with the target and qualifier ions used to identify and quantitate the analytes. Retention times are also presented.

TABLE 1. Target PAH Analytes and Internal Standards.

Compound	Target and Qualifier Ions	Retention Time (min)
Naphthalene	128, 129, 127	14.46
Naphthalene-d_8	136, 68	14.42
Naphthalene	128, 129, 127	14.46
Methyl naphthalene-d_{10}	152	16.29
2-Methyl naphthalene	142	16.14
1-Methyl naphthalene	142	16.36
Biphenyl-d_{10} [a]	164	17.47
Biphenyl	154	17.51
2,6-Dimethyl naphthalene	156	17.65
Acenaphthene-d_{10}	162,164,160	18.59
Acenaphthylene	152, 153, 151	18.24
Acenaphthene	153, 154, 152	18.66
2,3,5-Trimethyl-naphthalene	170	19.89
Fluorene-d_{10}	176, 174, 177	19.83
Fluorene	166, 165, 167	19.90
Phenanthrene-d_{10}	188, 94, 90	22.09
Phenanthrene	178, 179, 177	22.14
Anthracene-d_{10} [a]	188	22.21
Anthracene	178, 177, 179	22.26
1-Methyl phenanthrene	192,	23.62
Fluoranthene-d_{10}	212, 106	24.92
Fluoranthene	202, 203	24.96
Pyrene-d_{10}	212, 106	25.53
Pyrene	202, 200	25.45
Chrysene-d_{12}	240, 120, 236	28.35
Benz[a]anthracene-d_{12} [a]	240	28.66
Benz[a]anthracene	228, 229, 227	28.70
Chrysene	228, 229, 227	28.79
Benzo[b]fluoranthene-d_{12}	264, 132	32.12
Benzo[b]fluoranthene	252, 253, 126	32.21
Benzo[k]fluoranthene-d_{12}	264, 132	32.24
Benzo[k]fluoranthene	252, 253, 126	32.32

(cont'd)

TABLE 1 (cont'd).

Compound	Target and Qualifier Ions	Retention Time (min)
Benzo[e]pyrene-d_{12} [a]	264, 132	33.24
Benzo[e]pyrene	252, 126	33.36
Benzo[a]pyrene-d_{12}	264, 132	33.47
Benzo[a]pyrene	252, 253, 126	33.47
Perylene-d_{12}	264, 265, 260	33.80
Perylene	252, 126	33.92
Indeno[1,2,3-cd]pyrene-d_{12}	288	37.16
Indeno[1,2,3-cd]pyrene	276, 275, 138	37.21
Dibenz[ah]anthracene-d_{14}	292	37.25
Dibenz[ah]anthracene	278, 279, 139	37.34
Benzo[g,h,i]perylene-d_{12}	288, 144	37.84
Benzo[g,h,I]perylene	276, 275, 138	37.91

[a] Recovery standard

IV. Results and Discussion

A. Chemical Analyses

1. Particulate matter PAH

The particulate matter samples were extracted and analyzed as described in the Methods section. The analyses were conducted to also provide quantitation from UC Davis (UCD) and SwRI laboratories using different methods. The emission rate of PAHs, reported as micrograms per brake horsepower-hour (µg/brhp-hr), are summarized in **Table 2**. The PAHs from benz(a)anthracene to benzo(g,h,i)perylene were present predominantly on particles, whereas the compounds listed from naphthalene to anthracene were predominantly present in the vapor-phase. A fraction of the vapor-phase PAH however, were also present as reported in **Table 2**. Three fuel types were analyzed, including 100% REE, 50% REE, and 100% diesel. For benz(a)anthracene and chrysene, tested with or without the catalyst, there were lower emission rates for the 100% REE fuel than for the 50% REE blend or 100% diesel fuels. Benzo(b)-fluoranthene and benzo(k)fluoranthene appear to have similar emission rates for the 100% REE and 100% diesel fuels tested with or without the catalyst. The emission rates for benzo(e)pyrene, benzo(a)pyrene, and benzo(g,h,i)perylene and tested without the catalyst, were similar for 100% REE and the 50% blend, but were higher than 100% diesel. The emission rates for the particle-associated PAH are illustrated in **Figures 2 and 3** for the engine equipped with and without the catalyst, respectively. In general, for the particle-associated PAHs from benz(a)anthracene to benzo(g,h,i)perylene, the emission rates for 100% REE and 50% REE with catalyst are approximately one-half the emission rates without the catalyst.

The chemical analysis for the particle-associated PAHs as conducted by SwRI is summarized in **Table 3**. Particle filter samples were from the same set of transient cycles used for the particle samples analyzed by UCD. For all the particle-associated PAHs from benz(a)anthracene to benzo(g,h,i)perylene, the emission rates and patterns between fuels were similar to those observed by UCD. For example, the emission rate for chrysene increases as the amount of REE decreases and reaches the highest levels when using 100% diesel fuel (with or without the catalyst).

Ten particulate-associated PAHs were common in the analyses by SwRI and UCD laboratories. These PAHs include benz(a)anthracene, chrysene, benzo[b]fluoranthene, benzo[k]-fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-c,d]pyrene, dibenz[a,h]-anthracene, and benzo[g,h,i]perylene. The emission rates determined by UCD and SwRI and the trends for the fuels tested were similar for certain particulate-associated PAHs. For example, the emissions were very similar for benz(a)anthracene, where the emissions without catalyst for 100% REE, 50% REE and 2-D were 0.68, 1.11, and 1.03 µg/brhp-hr, respectively, as determined by UCD. In comparison, the benz(a)anthracene emission rates for 100% REE, 50% REE, and 100% 2-D fuels at SwRI were 0.8, 1.3, and 1.0 µg/brhp-hr, respectively. However, benzo(a)pyrene emissions were different between the two labs. For example, the 100% REE, 50% REE and 100% 2-D emission rates were 0.44, 0.41, and 0.24, respectively, as determined by UCD while, the emission rates were 0.8, 0.9, and 0.3 as determined by SwRI.

Table 2. Particulate-phase PAH emission rates from filter samples that were extracted and analyzed by UCD.

Compound	Catalyst?	PAH Emission Rate (µg / brhp-hr) 100% REE	50% REE	100% 2-D
Naphthalene	No	0.59	0.57	0.51
	Yes	0.49	0.36	0.41
Acenaphthylene	No	0.11	0.12	NQ
	Yes	0.10	0.07	NQ
Acenaphthene	No	NQ	NQ	NQ
	Yes	NQ	NQ	0.23
Fluorene	No	0.11	0.19	0.11
	Yes	ND	0.09	ND
Phenanthrene	No	1.04	1.63	1.91
	Yes	1.08	1.49	2.29
Anthracene	No	0.13	0.17	0.17
	Yes	0.12	0.08	ND
Fluoranthene	No	2.09	2.19	1.21
	Yes	1.52	1.79	1.37
Pyrene	No	3.58	3.97	2.89
	Yes	2.23	2.40	1.93
Benz(a)anthracene	No	0.68	1.11	1.03
	Yes	0.27	0.32	0.48
Chrysene	No	0.93	1.44	1.41
	Yes	0.33	0.45	1.09
Benzo[b]fluoranthene	No	0.68	0.62	0.61
	Yes	0.28	0.25	0.23
Benzo[k]fluoranthene	No	1.06	1.29	1.02
	Yes	0.34	0.09	0.35
Benzo[e]pyrene	No	0.49	0.55	0.34
	Yes	0.16	0.19	0.15
Benzo[a]pyrene	No	0.44	0.41	0.24
	Yes	0.16	0.14	0.09
Perylene	No	0.07	0.14	0.00
	Yes	ND	0.04	0.03
Indeno[1,2,3-c,d]pyrene	No	0.27	0.32	0.19
	Yes	0.16	0.11	0.11
Dibenz[a,h]anthracene	No	ND	ND	0.08
	Yes	ND	ND	ND
Benzo[g,h,i]perylene	No	0.66	0.55	0.31
	Yes	0.32	0.29	0.17

ND = not detected, NQ = not quantifiable.

Table 3. Particulate-phase PAH emission rates reported by SwRI [a]

Compound	Catalyst?	PAH Emission Rate (µg / brhp-hr) 100% REE	50% REE	100% 2-D
Benz(a)anthracene	No	0.8	1.3	1.0
	Yes	0.3	0.4	0.4
Chrysene	No	1.2	1.7	1.6
	Yes	0.4	0.6	0.8
Benzo[b]fluoranthene	No	1.1	1.2	0.8
	Yes	0.5	0.6	0.2
Benzo[k]fluoranthene	No	1.0	1.2	0.6
	Yes	0.4	0.5	0.1
Benzo[e]pyrene	No	0.9	1.1	0.5
	Yes	0.4	0.5	ND
Benzo[a]pyrene	No	0.8	0.9	0.3
	Yes	0.5	0.4	ND
Perylene	No	NR	NR	NR
	Yes	NR	NR	NR
Indeno[1,2,3-c,d]pyrene	No	ND	0.4	0.4
	Yes	ND	0.3	0.2
Dibenz[a,h]anthracene	No	ND	ND	ND
	Yes	ND	ND	ND
Benzo[g,h,i]perylene	No	0.8	0.6	0.1
	Yes	0.6	0.5	0.2

[a] Sharp *et al.*, (1996).
ND = not detected.
NR = not reported.

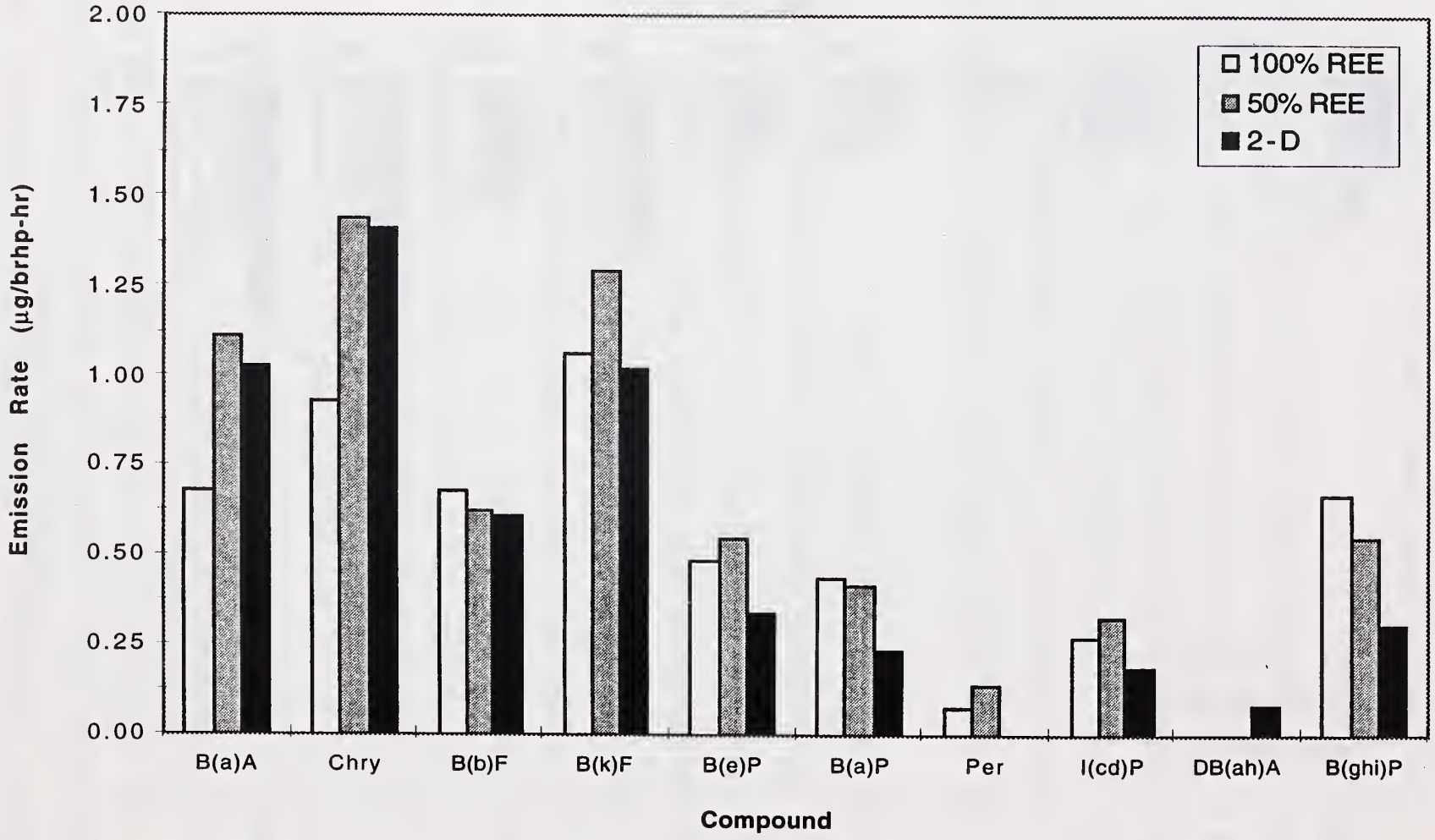

Figure 2. Emissions of particulate-associated PAHs for 100% REE, 50% REE, and 100% 2-D fuels without catalyst.

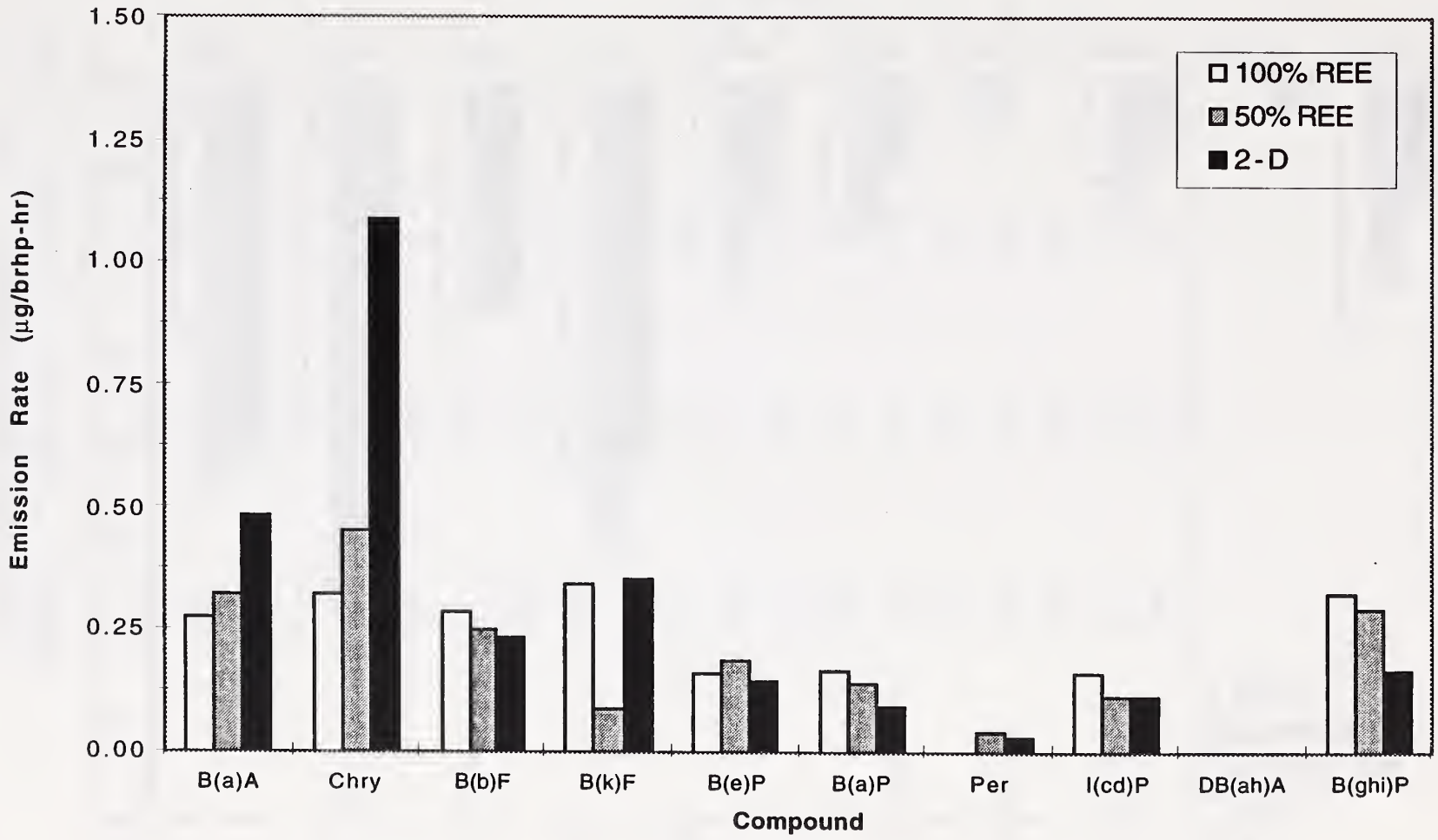

Figure 3. Emissions of particulate-associated PAHs for 100% REE, 50% REE, and 100% 2-D fuels with catalyst.

2. Vapor-Phase PAH

Vapor-phase PAHs were trapped with polyurethane foam (PUF) and were obtained from SwRI. The PUF samples were extracted using supercritical fluid extraction and chemically analyzed by GC/MS at UCD. The PAHs found on PUF samples obtained with and without catalyst were quantitated as summarized in **Tables 4** and **5**. The PAHs found on PUF samples obtained with and without catalyst have also been summarized in **Figures 4 and 5**. As the percentage of biodiesel fuel decreased, the vapor-phase PAH emission rate increased. For example, the emission rates for phenanthrene without the catalyst were 43.6, 92.8, 112.5, and 161.9 µg/brhp-hr for the 100% REE, 50% REE, 20% REE and 100% 2-D, respectively. The emission rates for fluorene, 2,3,5-trimethylnaphthalene, biphenyl and the methyl naphthalenes also increased with increasing percentage of diesel fuel. The emission rates of other vapor-phase PAHs such as anthracene, fluoranthene, and pyrene appear to be lower for 20% REE than for either 50% REE or 100% 2-D fuels.

The vapor-phase PAHs from naphthalene to pyrene were emitted at lower rates when the engine was equipped with a catalyst (**Table 5**). When using a catalyst with 100% REE fuel, there was an approximate 40% decrease in the vapor-phase PAH emissions compared to 100% 2-D fuel, and an approximate 50% decrease in vapor-phase PAH emissions when using the 100% RME fuel compared to the 100% 2-D fuel.

Unusually high levels of chrysene, benzo(b)fluoranthene, and perylene, typically associated only with particulate matter, were present in these PUF samples. These higher molecular weight PAHs are rarely found in vapor-phase PUF samples. For example, as seen in **Table 5**, unusually high levels of chrysene, benzo(b)fluoranthene, and perylene were measured. In many cases, the levels were higher in the PUF sample than in the matching filter particulate sample. A number of factors could contribute to this unusual result. One possibility is that the 20" x 20" filter could have been compromised, or torn during sampling, allowing particulate matter to be collected onto the PUF sample. However, based on the PAH levels measured on the filter particulate matter, the PAH levels previously measured on particulate matter, and the flow rates used to collect the vapor-phase PAHs, breakthrough of particulate matter onto the PUF could not account for the high PAH concentrations observed on the PUF.

Another possibility is that these PAHs were used as internal standards and were spiked onto the PUF for recovery data. According to SwRI, high levels of higher molecular weight PAHs were present on the 100% 2-D PUF samples, but it was not known if these PAHs were used for the majority of PUF samples sent to UCD. Spiking of PUF at UCD involved deuterated PAHs only. The high levels of the higher molecular weight PAHs quantitated in the SwRI PUF samples could not be resolved at this time.

The vapor-phase PAHs extracted from PUF and quantitated by SwRI are reported in **Table 6**. As the percentage of biodiesel fuel decreased, the vapor-phase PAH emission rate increased. For the PAHs naphthalene through phenanthrene, increased emission rates

were observed as the percentage of REE fuel was decreased. For anthracene, fluoranthene and pyrene, the emission rates did not increase dramatically. The emission rates for all vapor-phase PAHs measured were lower with the use of catalyst.

Table 4. Vapor-phase PAH emission rates without catalyst from PUF samples extracted and analyzed by UCD.

	PAH Emission Rate (µg/brhp-hr)			
Compound	100% REE	50% REE	20% REE	100% 2-D
Naphthalene	309.47	471.92	582.00	436.35
2-methylnaphthalene	267.00	486.95	728.72	821.36
1-methylnaphthalene	150.99	345.48	438.92	503.84
Biphenyl	86.27	197.91	304.36	402.74
2,6-dimethylnaphthalene	116.81	336.97	373.86	462.55
Acenaphthylene	38.33	64.85	67.11	64.96
Acenaphthene	51.38	93.96	148.88	172.20
2,3,5-trimethylnaphthalene	37.42	77.67	113.40	155.35
Fluorene	15.68	42.99	51.30	57.27
1-methylphenanthrene	9.00	54.44	23.89	35.45
Phenanthrene	43.59	92.81	112.49	161.88
Anthracene	4.45	51.08	13.53	84.64
Fluoranthene	5.66	11.25	5.12	5.65
Pyrene	7.48	13.03	8.64	9.93
Benz[a]anthracene	ND	47.30	1.48	1.85
Chrysene	15.98	11.98	2.96	123.21
Benzo[b]fluoranthene	22.55	6.31	5.12	293.07
Benzo[k]fluoranthene	ND	14.82	ND	438.59
Benzo[e]pyrene	3.94	0.32	ND	ND
Benzo[a]pyrene	ND	32.16	ND	ND
Perylene	ND	21.23	14.79	1864.21
Indeno[1,2,3-c,d]pyrene	ND	30.69	ND	ND
Dibenz[a,h]anthracene	ND	37.52	ND	ND
Benzo[g,h,i]perylene	ND	44.99	ND	ND

PAHs in bold face are typically found only in the particulate phase and not typically on PUF samples. Further, these levels are higher than the matching particulate samples.
ND = not detected.

Table 5. Vapor-phase PAH emission rates with catalyst from PUF samples extracted and analyzed by UCD.

	PAH Emission Rate (µg/brhp-hr)		
Compound	100% REE	50% REE	20% REE
Naphthalene	240.31	338.77	356.74
2-methylnaphthalene	170.11	268.26	391.65
1-methylnaphthalene	84.78	186.12	204.03
Biphenyl	49.32	134.13	226.19
2,6-dimethylnaphthalene	82.69	107.81	145.71
Acenaphthylene	17.59	17.46	22.37
Acenaphthene	32.09	77.25	97.52
2,3,5-trimethylnaphthalene	38.02	27.38	45.26
Fluorene	10.67	13.36	22.26
1-methylphenanthrene	22.06	5.56	7.32
Phenanthrene	26.07	34.79	60.83
Anthracene	22.88	0.00	3.87
Fluoranthene	7.02	3.04	2.51
Pyrene	5.65	2.65	2.51
Benz[a]anthracene	ND	ND	ND
Chrysene	14.68	3.31	3.03
Benzo[b]fluoranthene	12.12	4.76	5.85
Benzo[k]fluoranthene	ND	ND	ND
Benzo[e]pyrene	ND	ND	ND
Benzo[a]pyrene	ND	ND	ND
Perylene	38.38	7.14	10.56
Indeno[1,2,3-c,d]pyrene	ND	ND	ND
Dibenz[a,h]anthracene	ND	ND	ND
Benzo[g,h,i]perylene	ND	ND	ND

PAHs in bold face are typically found only in the particulate phase. The levels detected in the PUF were higher than in the matching particulate samples.
ND = not detected.

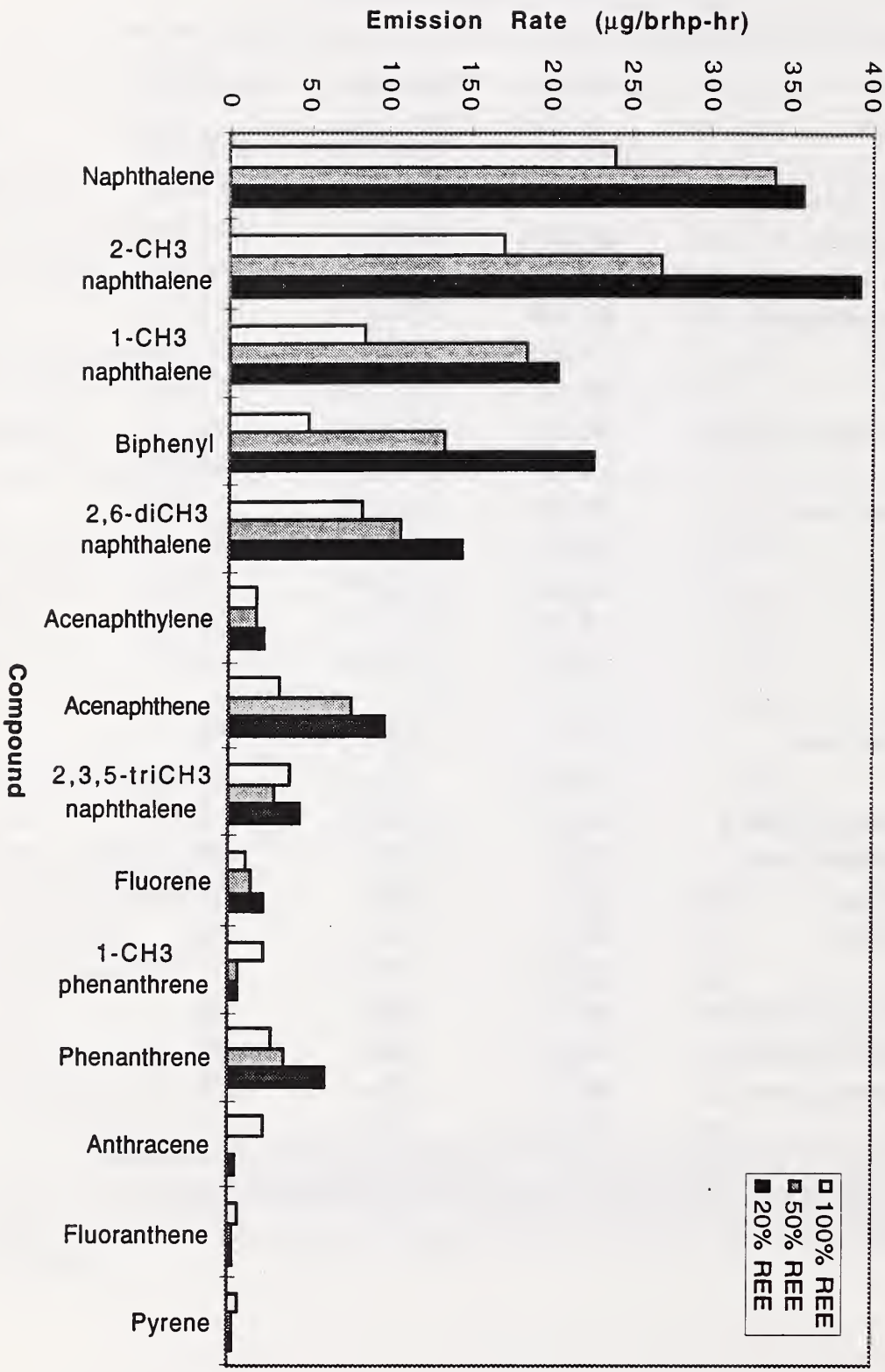

Figure 4. Emissions of vapor-phase PAHs for REE fuels with catalyst.

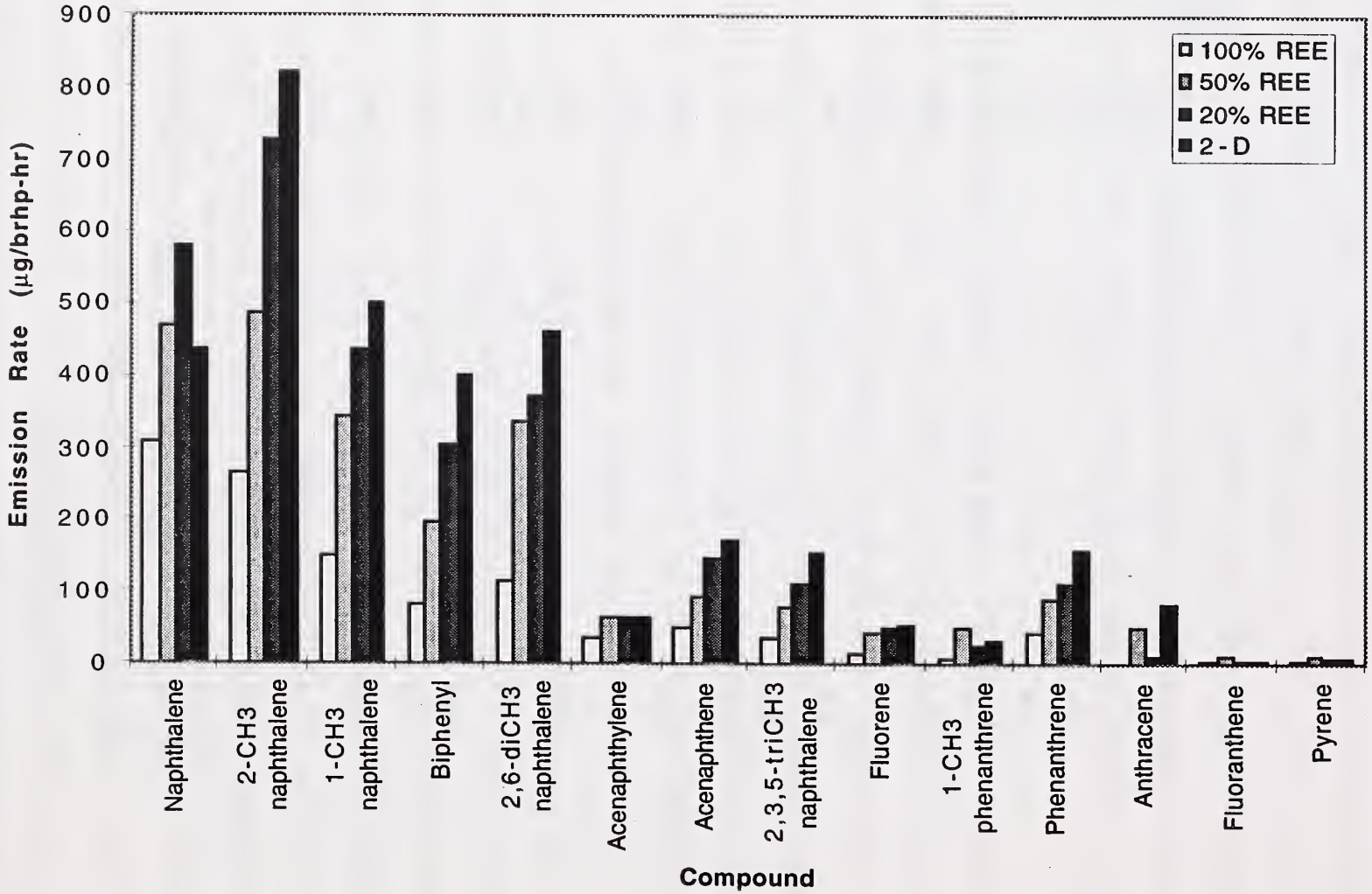

Figure 5. Emissions of vapor-phase PAHs for REE fuels without catalyst.

Table 6. Vapor-phase PAH emission rates from PUF samples reported by SwRI.

Compound	Catalyst?	PAH Emission Rate (μg/brhp-hr) 100% REE	50% REE	100% 2-D
Naphthalene	No	312	371	354
	Yes	173	288	355
Acenaphthylene	No	52	59	83
	Yes	9	16	30
Acenaphthene	No	ND	ND	ND
	Yes	ND	ND	ND
Fluorene	No	25	45	109
	Yes	11	15	53
Fluorenone	No	22	36	76
	Yes	14	22	100
Phenanthrene	No	71	103	226
	Yes	36	41	162
Anthracene	No	5	7	13
	Yes	2	1	4
Fluoranthene	No	126	134	122
	Yes	62	71	61
Pyrene	No	19	23	30
	Yes	7	9	11

ND = not detected.

B. Bioassay Analyses - Particulate Matter

Bioassays were conducted on sample extracts from particulate matter that was collected on filters from emissions without catalyst connected. The specific mutagenic activities of the particle extracts collected from 100% REE, REE blends, 100% RME, RME blends, and 2-D fuel emissions were determined. Specific mutagenic activity refers to the number of revertants (cells that have mutated) per microgram of particulate matter extracted from the filter samples. This value is determined from the slope of the linear portion of the dose-response curve. All filter extracts were tested for mutagenicity using the microsuspension bioassay procedure with tester strain TA98, with and without the addition of metabolic enzymes. The dose-response curves for the emissions from REE or RME fuels and blends are illustrated in **Figures 6, 7, 8,** and **9.** The specific mutagenic activity was lowest for emissions from both 100% REE and 100% RME fuels. The specific mutagenic activity increased as the percentage of biodiesel fuel decreased from 100% to 20% and was highest for emissions from the 100% 2-D fuel (0% biodiesel).

The general slope of the dose-response curves for all fuels were linear and the linear portions of the curves were used to determine specific mutagenic activity. The mutagenic activity of the particulate matter from the 20% REE blend has a potency similar to the 100% 2-D fuel based on particulate mass. The 20% RME blend had a slightly lower specific mutagenic activity than the 100% 2-D fuel emissions. The resultant emission rates for mutagenicity reflect these specific mutagenic activities.

Emission rates for mutagenic activity were calculated using the specific activity (rev/μg particulate matter) and the emission rate for particulate matter (g/brhp-hr). The resulting "mutagen emission rate" for both fuels increased with increasing percentage of reference diesel fuel added to biodiesel fuel. The results are summarized in **Figures 10** and **11** for the REE and RME fuels with metabolic enzymes added. For example, for the 100% REE fuel with metabolic enzymes added, the emission rate was approximately 3.5 x 10^5 revertants/brhp-hr (average value for 2 experiments), while the emission rate for 100% 2-D was approximately 1.2 x 10^6 revertants/brhp-hr. The emission rate for mutagenic activity acquired without the addition of metabolic enzymes was slightly higher than the activity when enzymes were added. This may indicate that there are some mutagenic compounds such as nitro-PAHs that may be present in the particulate matter from biodiesel fuel combustion.

The mutagenic activity of emissions from the 100% REE and 100% RME fuels were approximately equal, as were the emissions from biodiesel blends for these fuels when the same pecentage blends are compared. This may suggest that the complex mixture of compounds formed during fuel combustion is similar in mutagenic activity for the two biodiesel fuel types and their corresponding blends. Emission rates for the PUF samples are not reported, since all PUF samples contained breakthrough of "heavy" PAHs, which are typically found on filter particulate samples at lower concentrations.

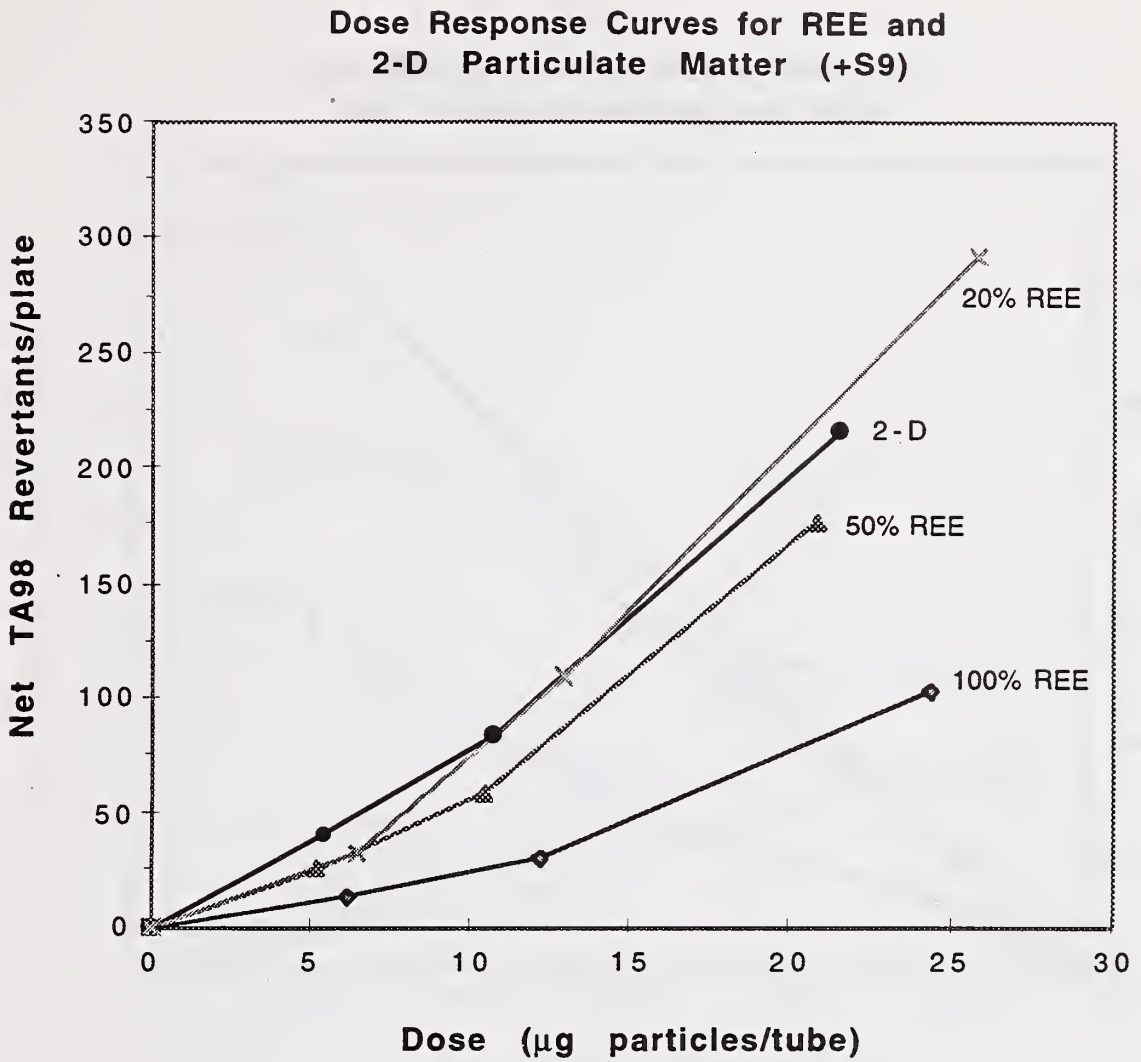

Figure 6. Dose-response curve for particulate matter from the100% REE, 50% REE, 20% REE, and 100% 2-D fuels with metabolic activation (+S9).

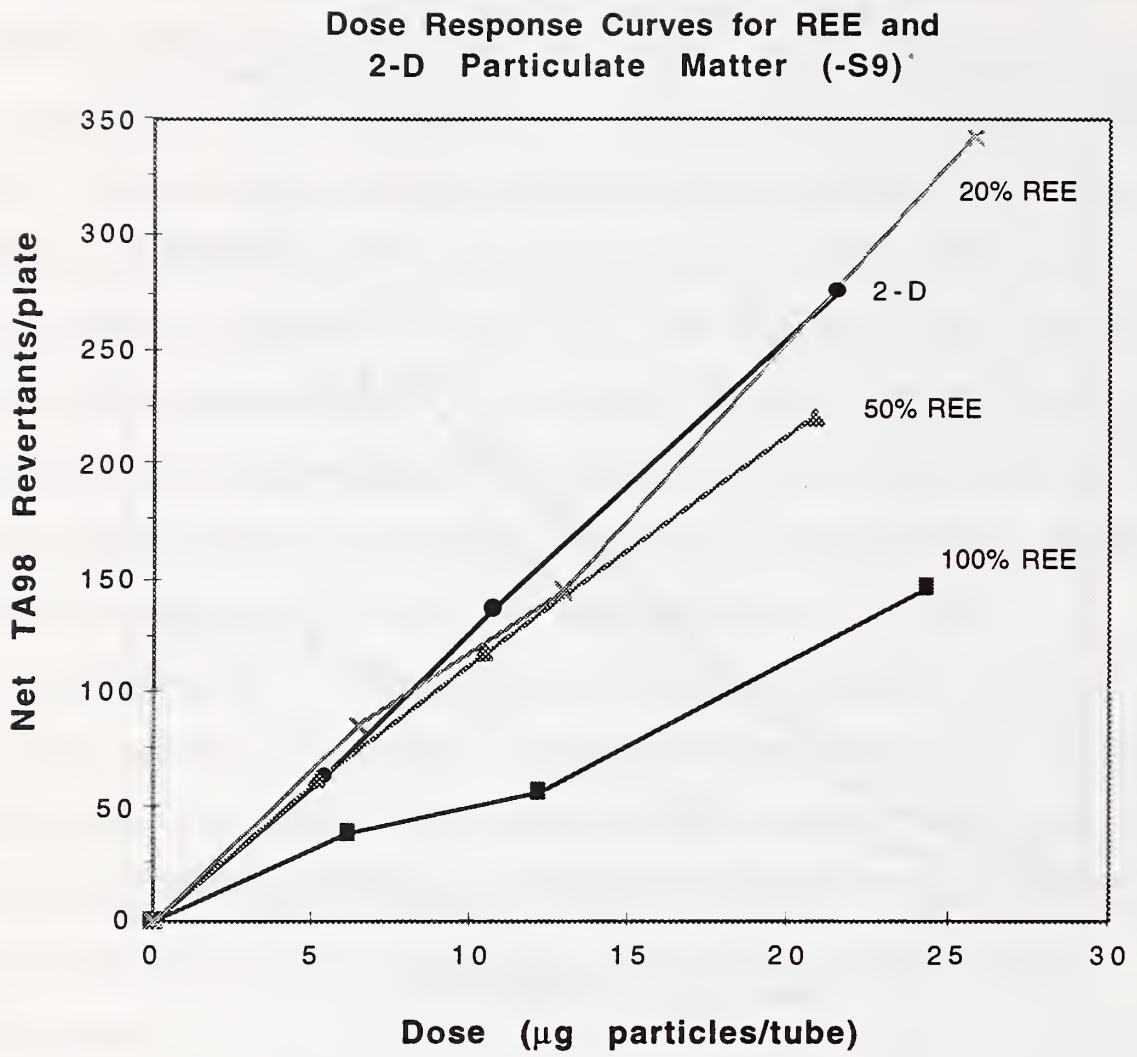

Figure 7. Dose-response curve for particulate matter from the 100% REE, 50% REE, 20% REE, and 100% 2-D fuels without metabolic activation (-S9).

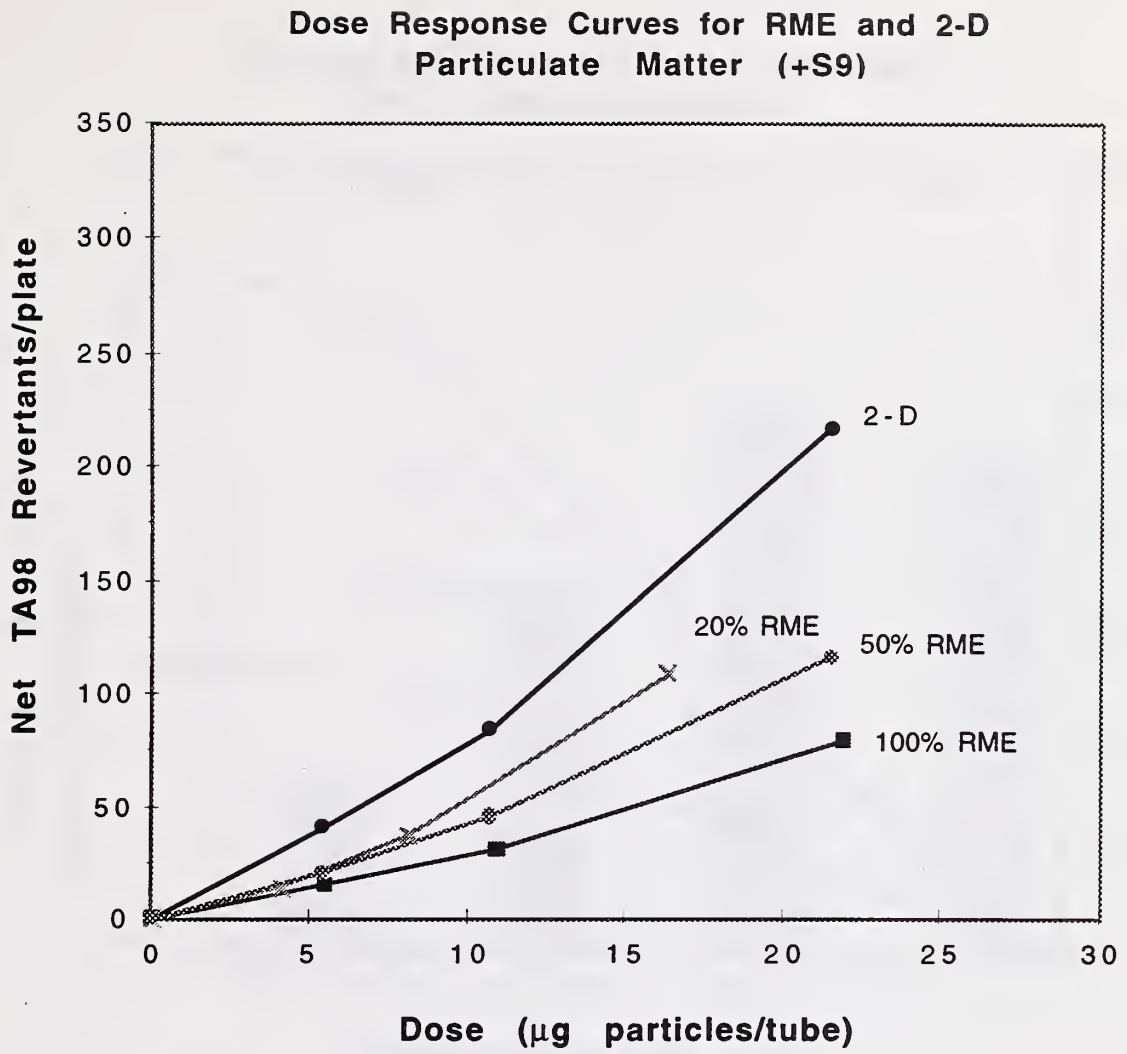

Figure 8. Dose-response curve for particulate matter from the 100% RME, 50% RME, 20% RME, and 100% 2-D fuels with metabolic activation (+S9).

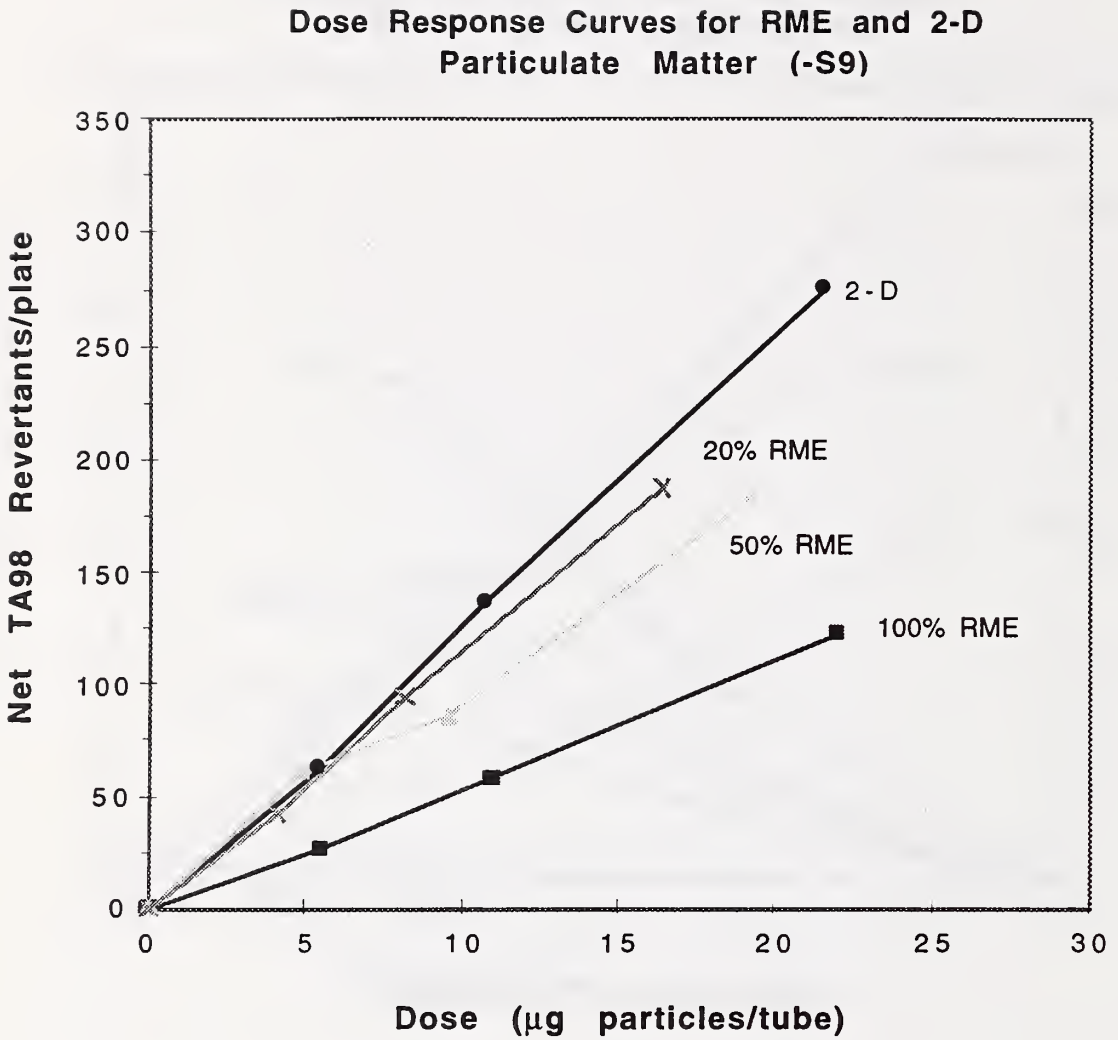

Figure 9. Dose-response curve for particulate matter from the 100% RME, 50% RME, 20% RME, and 100% 2-D fuels without metabolic activation (-S9).

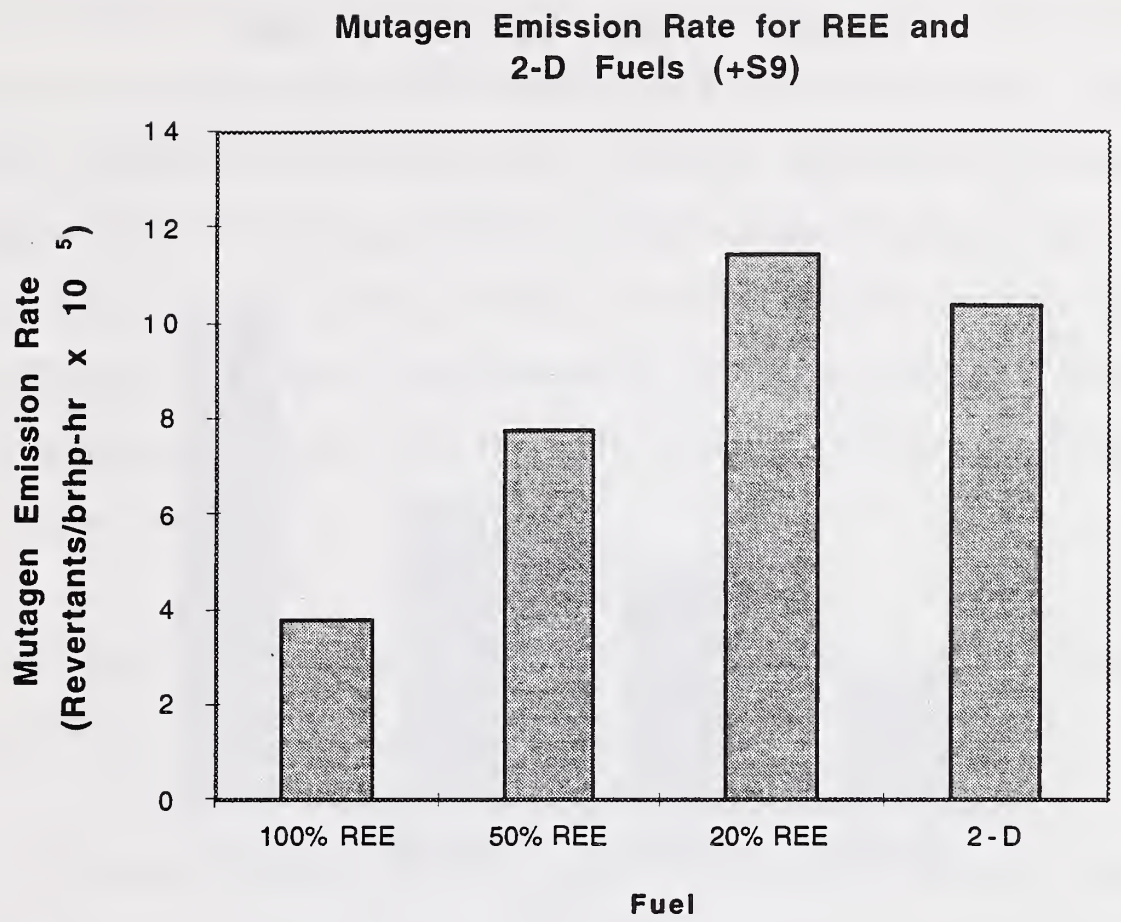

Figure 10. Particulate matter mutagen emission rates for REE and REE-blended fuels (+S9).

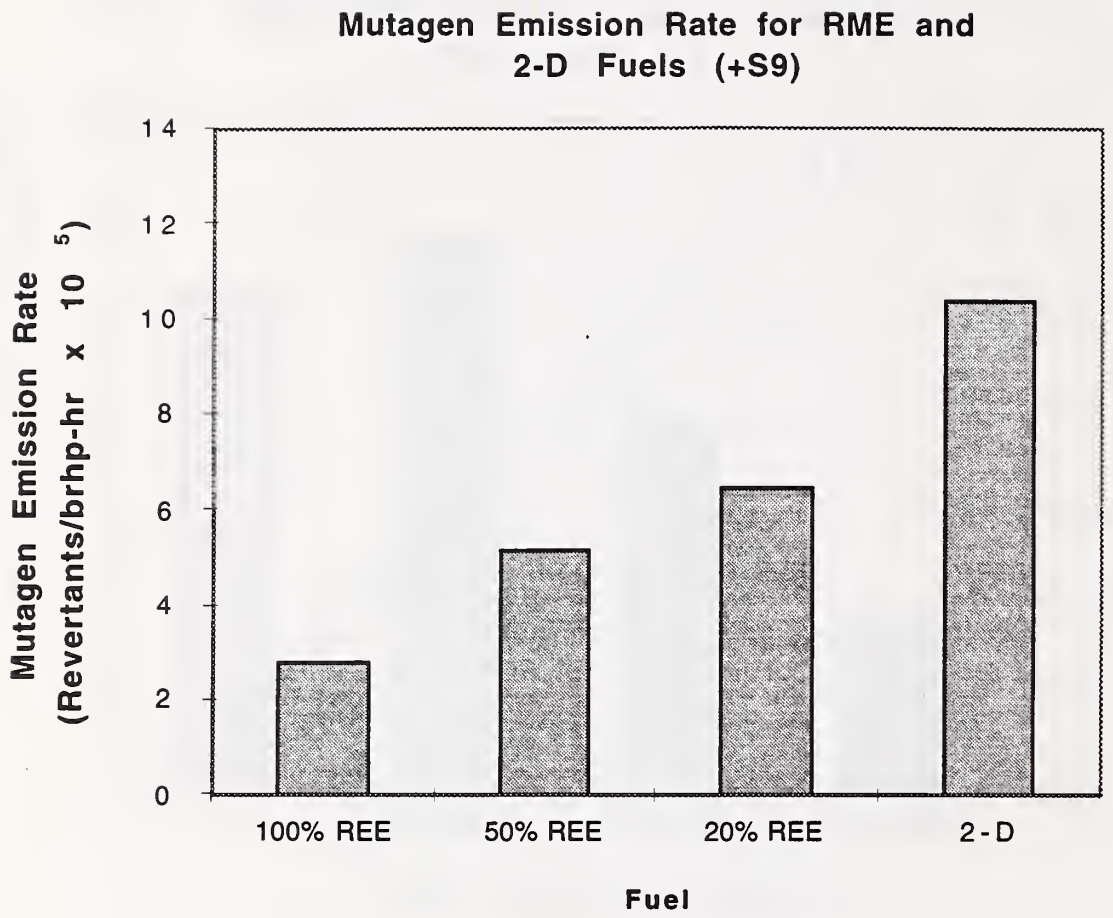

Figure 11. Particulate matter mutagen emission rates for RME and RME-blended fuels (+S9).

V. Conclusions

The particulate- and vapor-phase emissions from a medium-duty diesel engine using REE and RME biodiesel fuels were investigated and compared to those obtained using 100% 2-D reference fuel. Extracts from particulate- and vapor-phase emission samples were chemically analyzed for PAHs using GC/MS and PAH emission rates were determined as micrograms of PAH per brake horsepower-hour (μg PAH/brhp-hr). Bioassay analyses were conducted using the *Salmonella* microsuspension assay and mutagen emission rates (revertants/brhp-hr) were determined.

Based on the results of this study, the following conclusions are presented:

1. Particulate sample emission rates for benz(a)anthracene and chrysene were lower for the 100% REE fuel than for the 100% 2-D fuel, with or without the catalyst.

2. Emission rates of benzo(b)fluoranthene were similar for the 100% REE, 50% REE, and 100% 2-D fuels. There were higher emissions of fluoranthene, pyrene, benzo(e)pyrene, benzo(a)pyrene, and benzo(g,h,i)perylene for the 100% REE than for the 100% 2-D fuel. The emission rates for benzo(e)pyrene, benzo(a)pyrene, and benzo(g,h,i)perylene were typically in the sub-microgram per brhp-hr, similar to emission rates reported by SwRI.

3. Vapor-phase PAH emissions (naphthalene through phenanthrene) collected on PUF increased as the percentage of biodiesel fuel decreased.

4. High amounts of selected high molecular weight PAHs present in the PUF samples are typically found only in the particulate-phase. The source of these compounds is unknown, but appear to be from standards added to the matrix before sampling (refer to footnote a).

5. Vapor-phase PAH emissions were decreased overall by use of a catalyst, and were lowest overall for the 100% REE fuel.

6. The 100% REE and 100% RME had mutagen emission rates that were approximately three times less than those for the 100% 2-D fuel. The mutagen emission rates for the blends of biodiesel with diesel were between the rates for the 100% biodiesel and 100% 2-D fuels.

[a] Initially, the source of the heavy PAHs on the PUF samples were thought to be from "breakthrough" of sample where particulate matter with PAHs would pass through the filter and would be collected by the PUF. Southwest Research Institute has noted that they used a single 20" x 20" filter for this study. However, the levels of PAHs detected in the PUF samples are more consistent with an addition of PAH standards or chance contamination. First, the levels of many of these heavy PAHs were many-fold or orders of magnitude higher than those detected in the filter samples. Second, if breakthrough was occurring, the series of heavy PAHs present in the particle-phase would be expected to be present in the PUF samples at the ratios found in the particle phase. However, only a limited number of the heavy PAHs were detected at very high levels with some not present or detectable. Finally, detection limits for any of the heavy PAHs quantitated in the PUF were not a problem since their levels were in many cases considerably higher than identical PAHs detected in the filter samples.

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